

MEMORANDUM

SUBJECT: Construct Hazard Assessment for MCANs J15- 34 [REDACTED]

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I. INTRODUCTION

A consolidated Microbial Commercial Activity Notice (MCAN) for intergeneric constructs of *Saccharomyces*, designated as [REDACTED], has been submitted for review. The intergeneric strains will be used for [REDACTED] ethanol production. The production organisms will contain and express genes for [REDACTED]. While the submitter has identified the recipient strain as *S. cerevisiae*, in the Taxonomic Identification report for J15-34-35, Segal concluded that the identification of the subject microorganisms cannot definitively be assigned to *S. cerevisiae* and may instead be *S. boulardii* (1). However, if regarded as a variety of this species, "*boulardii*" strains fall within the larger taxon.

S. cerevisiae is a well-characterized eukaryotic fungus that is found worldwide. *S. cerevisiae* is a budding yeast that undergoes sexual or asexual reproduction depending on environmental conditions and is a model organism used in molecular biology studies. It is present in some foodstuffs and is used in various fermentation processes. *S. cerevisiae* can be an opportunistic pathogen, but the organism is generally recognized as safe (2).

II. GENETIC CONSTRUCTION OF THE PRODUCTION STRAIN

Genetic modifications of the recipient strain, described in detail by Penalva-Arana in the Genetic Construction Report for J15-34 and [REDACTED], are aimed at enhancing ethanol production [REDACTED] (3).

██████████ The production strains' ██████████ is used as a selectable marker.

III. POTENTIAL HAZARDS POSED BY THE GENETIC MODIFICATIONS

A. Inserted Genes

The proposed genetic modifications to the recipient strain of *Saccharomyces* to create the two different constructs are described in the Genetic Construction Report (3). [REDACTED]

The intergeneric sequences are widespread in the environment and are associated with well characterized cellular metabolic pathways. These sequences do not encode any mobilization functions.

B. Cellular Effects of Modifications of Inserted Sequence

Myriad reports describe metabolic engineering studies where yeast strains are used as production organisms. It is unlikely that the proposed modifications will confer any selective advantages to the production strains. According to the MCAN, the phenotypes of the production strains do not vary significantly from the parental strains with the exception of [REDACTED]

C. Stability of Inserted Genes

There is low hazard associated with the proposed phenotypes and the genetic modifications made to the *Saccharomyces* recipient strain. The modifications are expected to be stable given that the insertions are chromosomal.

IV. POTENTIAL FOR HORIZONTAL GENE TRANSFER

No transfer or mobilization functions were introduced into the production strains. While horizontal gene transfer (HGT) from bacteria and among fungi has been well documented, evidence suggests that HGT rates into and between fungi are low (4). Therefore, it is unlikely that the intergeneric sequences would be transferred to other organisms.

V. CONCLUSIONS

There is little concern for the genetic modifications proposed to the recipient strain to arrive at the different potential production strains of *Saccharomyces*; there are low hazards associated with the intergeneric sequences. The potential for gene transfer from the proposed production microorganisms to other yeasts in the environment expected to be low given chromosomal insertion of the desired genes and low HGT rates in yeast. In summary, there is little concern associated with the genetic modifications used to create the proposed *Saccharomyces* strains for fermentation processes.

REFERENCES

1. Segal, Mark. 2015. Taxonomic Identification Report for J15-34/35. Office of Pollution Prevention and Toxics. Environmental Protection Agency, Washington, DC.
2. http://www.epa.gov/biotech_rule/pubs/fra/fra002.htm
3. Penalva-Arana. 2015. Genetic Construction Report for J15-34/35. Office of Pollution Prevention and Toxics. Environmental Protection Agency, Washington, DC.
4. Fitzpatrick, D.A. 2011. Horizontal gene transfer in fungi. *FEMS Microbiol. Letters* **329**(2012):1-8.